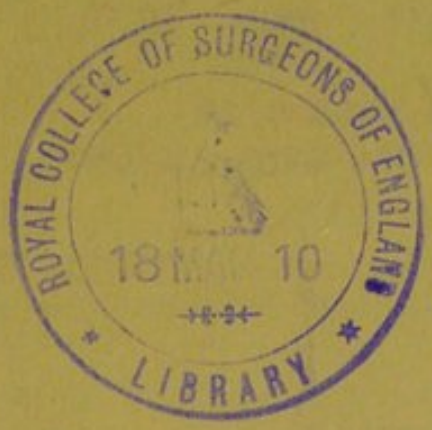
Examination of the blood : with examples of the wide range of its value / by Herbert French.

From "THE PRACTITIONER" *for March*, 1903.

EXAMINATION OF THE BLOOD: WITH EXAMPLES OF THE WIDE RANGE OF ITS VALUE.

BY HERBERT FRENCH, B.M. (OXON.), M.R.C.P. (LOND.),
Medical Registrar, Guy's Hospital; Radcliffe Travelling Fellow, Oxford.



"THE PRACTITIONER," LIMITED,
30, HOLBORN, LONDON, E.C.

LONDON:
PRINTED BY EYRE AND SPOTTISWOODE,
HIS MAJESTY'S PRINTERS,
EAST HARDING STREET, E.C.

From "THE PRACTITIONER" *for March*, 1903

EXAMINATION OF THE BLOOD: WITH EXAMPLES OF THE WIDE RANGE OF ITS VALUE.

BY HERBERT FRENCH, B.M. (OXON.), M.R.C.P. (LOND.),
Medical Registrar, Guy's Hospital; Radcliffe Travelling Fellow, Oxford.

A COMPLETE examination of the blood requires time: it also needs a certain expenditure upon special apparatus. For one, or both, of these reasons it is frequently neglected by the busy medical man. Nevertheless, the help it may afford in many cases of difficult diagnosis is so great that it seems worth while to give instances of cases which prove its practical value.

It is a prevalent belief that an examination of the blood is needed only in the so-called "Blood-diseases." In these, of course, such examination is essential. Thus a patient with an enlarged spleen is not necessarily suffering from spleno-medullary leuchæmia; a leucocyte-count may show no increase in the leucocytes, and the patient may, as a fact, have splenic anæmia. The distinction between these conditions is of great importance. In the former case, removal of the spleen by operation will almost inevitably be fatal; in the latter, such operation may be the means of curing the invalid (Osler).

Again, there may be a leucocytosis up to, perhaps, 50,000 per c.mm.; there may be a moderately big spleen, and it may be doubtful whether some enlarged glands in the neck may not be tuberculous. A diagnosis of early "leuchæmia" may perhaps be made; but this is incomplete. To perfect the diagnosis, a differential count of the leucocytes is needed. There may, for example, be 70 per cent. of small lymphocytes, or even more, indicating the lymphatic type of leuchæmia; or, on the other hand, 30 per cent., perhaps, of myelocytes, with less than 20 per cent. of small lymphocytes, so that the spleno-medullary variety must be diagnosed. The distinction, it may be urged, is of little value from the point of view of treatment; but it changes the prognosis entirely. In the

former case the patient will probably have but a few months to live ; in the latter he may survive three years or more.

In another case, with the enlarged spleen there may be no enlarged glands, and there may be a soft cardiac *bruit* and anæmia ; here it may be uncertain whether or not the *bruit* is hæmic, and doubts arise as to whether the patient is suffering from leuchæmia or from ulcerative endocarditis. In each there may be irregular pyrexia, so that the temperature-chart will not decide the question. A blood-count may show some leucocytosis, and yet the diagnosis remain undecided. A differential leucocyte-count may be the determining factor. An increased proportion of polymorpho-nuclear cells and an absence of myelocytes will prove the leucocytosis to be due to ulcerative endocarditis rather than to early leuchæmia.

Again, without an accurate estimation both of red corpuscles and of hæmoglobin, and conclusive proof that the hæmoglobin is less diminished than are the red corpuscles, it is impossible to be certain of the diagnosis of pernicious anæmia. Sometimes the cachexia of carcinoma ventriculi may strongly suggest pernicious anæmia until a blood-examination decides against it.

Even should the ratio of hæmoglobin to red corpuscles be of the type found in pernicious anæmia, the diagnosis may easily be wrong unless a differential leucocyte-count be also made. In ankylostomiasis the hæmoglobin-value is also greater than normal ; but in this condition there is a considerably increased percentage of the coarsely-granular eosinophile corpuscles, such as is not found in true pernicious anæmia. The treatment of the two conditions is very different ; anthelmintics and iron are required in the former state ; oral and gastro-intestinal disinfectants, extract of bone-marrow, and arsenic in the latter.

But it is not only in the "Blood-diseases" that examinations of the blood may be the turning-point in diagnosis, nor is it always the same part of a complete blood-examination which turns the scale in different cases. Sometimes the diagnosis may depend upon the total number of leucocytes ; sometimes upon the differential leucocyte-count ; sometimes upon the ratio of hæmoglobin to red corpuscles ; sometimes upon the total number of red corpuscles ; sometimes upon the specific gravity of the blood.

That this is not purely theoretical advice will best be shown by quoting actual cases.

I.—TOTAL NUMBER OF LEUCOCYTES.

First, and simplest, are *cases in which the total leucocyte-count has changed the diagnosis.*

In the following three instances the original diagnosis was typhoid fever; and, as they show, the fact that there is no leucocytosis, but rather a leucopenia, in enterica may frequently be made use of for diagnostic purposes.

(1) Florence A., aged 23, having been ill ten days with headache, feverishness, anorexia, and obscure abdominal pain, was sent to Guy's Hospital as suffering from typhoid fever, and admitted under the care of Dr. Hale White. Her temperature was 101·8° F., her pulse 95, and her respirations 16 per minute. At the same time that blood was taken for a Widal test, a blood-count was made, and 24,000 leucocytes per cubic millemetre were found. The differential count showed:—

Polymorpho-nuclear cells - -	80 per cent.
Small lymphocytes - - -	9 „
Transitional forms - - -	1·5 „
Large lymphocytes - - -	9 „
Coarsely-granular eosinophile cells -	0·5 „

The conclusion, therefore, was that the condition was not one of enterica, but rather of deep-seated suppuration. There were no pelvic symptoms, and the patient was unmarried; but there was considerable abdominal pain, and, following the lead given by the total and differential leucocyte-counts, laparotomy was performed in the middle line. Double pyo-salpinx was found, and the patient made a complete recovery after removal of the tubes.

(2) Mary W., aged 14, was perfectly well up to August 29, and on that day was sent home from school on account of severe headache. That night she was feverish, and was sick, but complained chiefly of her headache. She stayed in bed next day; on the day following she came into hospital, under the care of Dr. Hale White. Headache, pyrexia, and some general abdominal pain were her chief symptoms. The temperature was 101·2° F.; the respirations 22 per minute;

and the pulse 120. There was nothing definite to be made out in the abdomen; and on rectal examination no abnormality was discovered. It seemed to some that the girl must be suffering from typhoid fever, though the spleen was not palpable. It was too early for rose-spots to have appeared, or for the Widal reaction to be expected. A blood-count changed the diagnosis at once. It revealed the presence of 49,000 leucocytes per cubic millimetre, and typhoid fever was excluded. The abdominal pain suggested deep-seated pus as a cause for this leucocytosis. There being no localising symptoms, the abdomen was opened in the middle line; a deeply situated appendicular abscess was discovered, opened, and drained; and the patient made a complete recovery.

(3) Henry S., aged 43, was admitted under the care of Dr. Perry, having been ill a fortnight. During this time he had suffered from severe headache, a cold in his nose and throat, and pains in his loins; he had also noticed some lumps in his left groin. He had had no sore place on his leg, foot, or buttocks; had no discharge from his penis; and had had no rigor. The lumps in his groin appeared to be enlarged glands, but no cause for their enlargement could be discovered; they were not very tender, red or inflamed. His temperature was 102·4° F., his pulse 88, and his respirations 30 per minute. He thus exhibited the comparatively slow pulse-rate in proportion to his temperature, which is so common in typhoid fever; and he had, moreover, about a dozen rose-red spots upon his abdomen, strongly suggestive of enterica. His spleen was not palpable, nor had he bronchitis. He came in on September 10th, and his temperature fell by stages, reaching normal on September 15th. It remained normal until October 4th, during all which time he was treated as a case of typhoid fever. The glands in his groin showed little change. On October 4th the temperature rose again, and remained continuously raised until October 12th, as though there had been a relapse. The leucocytes had been counted on September 15th, when they were 9,700 per cubic millimetre. The next counts were:—

On October	6th	-	-	13,600	per cubic millimetre.	
,,	,,	7th	-	-	16,250	,, ,, ,,
,,	,,	8th	-	-	14,100	,, ,, ,,
,,	,,	9th	-	-	17,800	,, ,, ,,

On October	10th	- -	17,000	per	cubic millimetre.
„ „	11th	- -	17,500	„	„ „
„ „	12th	- -	14,200	„	„ „
„ „	13th	- -	11,718	„	„ „
„ „	20th	- -	12,000	„	„ „

Such definite leucocytosis argued strongly against enteric fever, and suggested pus retained under pressure. Nevertheless, though the Widal reaction was negative, so closely did the patient resemble one suffering from enterica, that it was only in November that this diagnosis was finally abandoned. By this time (seven weeks after patient's admission) the inguinal glands began to soften; the skin over them grew red, and a needle inserted into them withdrew pus, from which a pure culture of Staphylococcus pyogenes aureus was obtained. The abscess was incised and drained, and the patient got quite well.

Such instances of the valuable assistance afforded by counting the leucocytes in doubtful cases of typhoid fever, particularly when the symptoms are abdominal, might readily be multiplied.

Another group of cases in which the same means may be invaluable in deciding the diagnosis is that in which the *doubt lies between hepatic abscess and malaria.* This will naturally be of greatest service in the tropics; but not infrequently the difficulty arises in this country also, in patients who have returned home from abroad. As Rogers (*British Medical Journal*, 1902, Vol. II., p. 850) has found in many instances, a liver-abscess is accompanied by as decided a leucocytosis as 30,000 or 40,000 leucocytes per cubic millimetre; whereas malaria resembles typhoid fever in showing leucopenia, the leucocytes rarely numbering more than 5,000 per cubic millimetre; and a differential count reveals a high percentage of large lymphocytes. Hence, in a doubtful case, the simple process of making a total leucocyte-count may decide whether the treatment is to consist in the administration of quinine, on the one hand, or in an exploration of the liver upon the other.

II.—DIFFERENT VARIETIES OF LEUCOCYTES.

Secondly, examples may be given of *cases in which a differential count of the leucocytes has decided the diagnosis.*

(1) Frederick M., aged 28, was a married man, with young children entirely dependent on him. He had been for some years

in the Hoogley Pilot Service, from which he derived a good income, with the prospect of a pension in the course of time. Should he be invalided from the Service, ruin stared him in the face. Five years previous to his coming to Dr. Bryant for examination he caught a severe cold when on duty on a steamer, since which time his cough had never quite left him. Latterly he had been subject to peculiar paroxysmal attacks of coughing, which came on suddenly, and lasted variously from ten minutes to an hour. They recurred sometimes as often as two or three times in the twenty-four hours. They were at first treated as asthmatic, but without great benefit; and then, owing to the somewhat "brassy" character of the cough, the condition was variously thought to be one of thoracic aneurysm or of malignant growth. On this account he was given six months' furlough to return to England from the Hoogley, in order to confirm one diagnosis or the other. If tumour or aneurysm were the malady, he would be invalided from the Service; if it proved to be a less serious condition, he would retain his post. From the patient's point of view it was a most momentous question to decide. The fact is well known that a differential leucocyte-count in asthma, during the paroxysms or when they are frequent, often reveals a high proportion of the coarsely-granular eosinophile cells. A blood-examination was therefore made, with the following results :—

Red corpuscles	-	6,587,500 per cubic millimetre.
White corpuscles	-	10,625 „ „
Hæmoglobin	-	90 per cent.

The differential leucocyte-count, in which over 1,000 corpuscles were enumerated, was as follows :—

Small lymphocytes	-	13 per cent.
Large lymphocytes	-	4 „
Polymorpho-nuclear cells	-	24 „
Coarsely-granular eosinophile cells	-	*59 per cent.*

The films were most remarkable; of every two leucocytes one was a coarsely-granular eosinophile cell; under the oil-immersion lens as many as five or six such cells might be seen in the field at once. This was strong evidence in favour of asthma as the diagnosis. A very careful examination was further made before new growth and aneurysm were finally

excluded. The chest was emphysematous, and the heart-sounds inaudible. The lungs were everywhere resonant, the entry of air equal on the two sides, and there were no distended veins on the chest-wall; the patient was robust and strong. His radial pulses were equal, and he had no tracheal tugging. His pupils were equal, he had no pain; and he gave no history, and showed no evidence, of syphilis. He was skiagraphed, and no sign of growth or aneurysm could be seen. His voice was, he said, different to what it had been, so that he could no longer sing; but the laryngoscope showed no sign of a foreign body in the larynx, nor of a papilloma or malignant growth, nor of any paralysis of either vocal cord. His cough, as has been stated, was undoubtedly "brassy," and suggestive of thoracic aneurysm. Nevertheless, all the other signs being negative, and the differential count being very positive, it was largely on the strength of the greatly increased number of coarsely-granular eosinophile cells in his blood that the patient was able to retain his post in the Hoogley Pilot Service; and there has since been no reason for changing the diagnosis of asthma thus made.[1]

Actual examples are so much more impressive than the mere statement of a general fact, that the following may be given as illustrating quite another group of cases :—

(2) Nathaniel H., aged 20, had been on active service in South Africa. While there he had noticed nothing the matter with himself; but on his return to England he found that his feet began to swell, and from time to time he passed blood in his urine. When examined his urine contained bright blood and albumen (2 parts per 1,000). A few blood-shreds were found, which might possibly have been casts; and it was thought that he was suffering from a mild attack of Bright's disease. This necessitated treatment by rest in bed in flannel night-clothes, diaphoretics and purgatives, and a milk-diet. The patient, who had been a soldier, and who felt quite well in himself, naturally found a milk-diet and restraint in bed most irksome; and yet such treatment was essential if his kidneys

[1] The fact that the patient had been in India raises another question. His eosinophilia might have been due to the presence of some parasite. But he was not at all anæmic. He had more red corpuscles than normal. He was a gentleman; a European; and lived mostly on shipboard. These points are all against parasitic infection. Moreover, his fæces always appeared quite normal, though they were not particularly examined for parasitic ova.

were inflamed. A blood-count, however, supplied the following data:—

Red corpuscles	-	80 per cent.
White corpuscles	-	8,500 per cubic millimetre.
Hæmoglobin	-	70 per cent.

And a differential leucocyte-count showed:—

Small lymphocytes	-	18 per cent.
Large lymphocytes	-	10 ,,
Polymorphor-nuclear cells	-	56 ,,
Coarsely-granular eosinophile cells	-	*16 per cent.*

It is an established fact that many parasitic diseases are accompanied by an increase in the coarsely-granular eosinophile cells. This is the case, for example, in patients harbouring Ankylostomum duodenale, Filaria sanguinis hominis, Trichina spiralis, and Bilharzia hæmatobia. The above differential leucocyte-count strongly suggested that Bilharzia hæmatobia was the cause of the hæmaturia in this patient from South Africa, the œdema being consequent on his loss of blood. Bilharzia eggs were searched for in his urine, but none were found; nevertheless, on the strength of the blood-examination, the diagnosis of Bright's disease was relinquished. To the patient's great joy, full diet was allowed, and no ill-result followed. The hæmaturia, slighter in amount, continued up to the time of his discharge, but he felt quite strong and well, and was happy to know that he was not suffering from Bright's disease.

Not a few similar instances of obscure hæmaturia must have occurred since the war in South Africa began. It is not always easy to find the ova of Bilharzia in the urine. A differential leucocyte-count, revealing an increased proportion of coarsely-granular eosinophile cells, may give the clue to the correct diagnosis.

Another, and yet distinct, group of cases in which the differential leucocyte-count has been a great factor in assisting the diagnosis is that of the Cornish miners. Those who work in certain of the Cornish mines have, for four or five years past, been exceedingly anæmic. Recently, Dr. Haldane and Dr. Boycott (*Journal of Hygiene*, January, 1903) have investigated the nature of this malady, and have demonstrated that it is due to the presence of the Ankylostomum duodenale.

This might have suggested itself long ago, had even a moderately-careful examination of a stained blood-film been made. The differential leucocyte-counts vary widely in different patients, but in nearly every case there was most obvious eosinophilia. An average case gave the following figures :—

Small lymphocytes - - -	18 per cent.
Large lymphocytes - -	8 „
Polymorpho-nuclear cells - -	52 „
Coarsely-granular eosinophile cells -	*22 per cent.*

Such an increase in the coarsely-granular eosinophile cells suggested a parasitic disease. After the administration of thymol, the diagnosis was verified by the discovery of numbers of the actual worms in the fæces. The differential leucocyte-count, and the subsequent discovery of the worm, entirely altered the lines upon which the treatment of the patients should be carried out.

III.—ESTIMATION OF HÆMOGLOBIN.

Thirdly, there are *cases in which an accurate determination of the hæmoglobin-value of the corpuscles is essential.*

This is the case, of course, in every case of pernicious anæmia. Not infrequently, however, the colour of the patient may so strongly suggest pernicious anæmia that without the blood-determination a wrong diagnosis may easily be made. The blood-examination may be quite as valuable when it negatives a diagnosis as when it leads to a positive result. The following are two actual cases in point :—

(1) Joshua P., aged 46, was quite well ten months before examination. He then noticed that his skin began to get bright-yellow, and that he was short of breath when he worked as a coal-porter. A doctor saw him, and found that the yellow colour of his skin was not due to jaundice. There was a little wasting as time went on, but not much. There was no vomiting ; the bowels were frequently opened three times a day, and on several occasions there was melæna. There was a local cardiac systolic *bruit,* but nothing else could be discovered. The abdomen seemed normal, and a rectal examination revealed nothing. The patient seemed to be suffering from pernicious anæmia. Ten months after his

symptoms began he was in bed, too weak to work, but not cachetic. He was of a lemon-yellow colour, with blanched lips, gums, and hands. There was no jaundice, and he had not been sick once. His blood was examined, with the following result :—

Specific gravity	-	1,036.
Hæmoglobin	-	*12 per cent.* (by Haldane's hæmoglobinometer).
Red corpuscles	-	*44 per cent.*, or 2,235,000 per cubic millimetre.
White corpuscles	-	9,375 per cubic millimetre.

A differential leucocyte-count showed :—

Small lymphocytes	-	18 per cent.
Large lymphocytes	-	6 „
Polymorpho-nuclear cells	-	75 „
Coarsely-granular eosinophile cells	-	1 „

The hæmoglobin value of the red corpuscles was therefore $\frac{12}{44}$, much less than unity, instead of more, as it is in pernicious anæmia. The blood-film showed many poikilocytes, megalocytes, and microcytes, and, in counting 1,031 leucocytes, 7 nucleated red corpuscles were seen. Poikilocytosis, irregularities in the size of the corpuscles, and the presence of nucleated red cells, are distinctive of no single form of anæmia ; these changes occur in every form of anæmia, if it is severe. The diagnosis was, therefore, other than pernicious anæmia ; and a common cause of severe anæmia in middle-aged persons is gastric carcinoma. The patient had given no evidence of gastric symptoms ; but whilst he was kept under observation by Dr. Fawcett he developed abdominal pain, and rigidity of his right rectus abdominis muscle, with tenderness. He began to be sick—at first occasionally, later often—and the vomit contained no free hydrochloric acid. The blood-examination had been the means of making the diagnosis by excluding pernicious anæmia, which was so closely simulated in the earlier stages of gastric carcinoma.

(2) John D., aged 40, was admitted under the care of Dr. Hale White, having the outward appearance of a patient suffering from pernicious anæmia. He began to be "out of sorts" six months previously. His skin began to be bright-yellow in colour, and his feet and legs swelled ; three months

later he fell down when doing his work, and consulted a doctor. He was then weak and anæmic, without paralysis; no jaundice was present, but the skin was of a lemon-yellow tint, and there was some œdema of the legs. When admitted to hospital the condition was very similar. He was short of breath on exertion, but otherwise seemed well; and he was not wasted. His heart was of normal size, and there was a blowing systolic *bruit*, best heard in the third left space, but also heard in the aortic area, and at the point of impulse. The patient might well have been suffering from pernicious anæmia with hæmic *bruits*, but a blood-examination excluded this at once. There were found:—

Red corpuscles	-	*51 per cent.*, or 2,600,000 per cubic millimetre.
White corpuscles	-	5,500 per cubic millimetre.
Hæmoglobin	-	*28 per cent.* (by Haldane's hæmoglobinometer).

Far from being greater than unity, the hæmoglobin value of the red corpuscles was $\frac{28}{51}$, and pernicious anæmia was negatived. Streptococci were cultivated from the patient's blood, and the case turned out to be one of ulcerative endocarditis.

Instances of cases in which different diseases have at first strongly suggested pernicious anæmia might readily be multiplied. It is by means of a blood-examination that the mistake can be avoided.

IV.—ENUMERATION OF RED CORPUSCLES.

Fourthly, we have *cases in which counting the total red corpuscles is important to the diagnosis*. These include all cases of anæmia. It is by enumerating the red corpuscles that the improvement produced by any given form of treatment can best be gauged in each individual patient. But in a few other cases counting the red corpuscles has also assisted the diagnosis. For example:—

Percy D., aged 4, was admitted under the care of Dr. Bryant for dyspnœa and cyanosis. When he was a year old he began to have cough and attacks of dyspnœa and cyanosis; and he had had these attacks at frequent intervals. He had, however, become much worse three weeks before admission. He was now very cyanosed, and his fingers and toes were

clubbed. His condition strongly suggested congenital heart-disease, but upon examination of his heart no abnormality beyond a somewhat increased area of præcordial dulness could be detected. There was no cardiac *bruit* at all. His lungs were resonant, but râles and rhonchi were numerous all over them, and there was a possibility that the primary trouble was in the lungs themselves. The blood was examined, and the red corpuscles were *15,462,800 per cubic millimetre* when taken from the ear; and in another specimen from the middle of the back, where there was less congestion, there were found 14,025,000 per cubic millimetre. In many different forms of cyanotic congestion the total number of red corpuscles has been found increased, as, for example, in cases of mitral stenosis; also in persons who live at high altitudes; but of all conditions, congenital heart-disease is found to give the most marked increase. So great a rise as the above, up to three times the normal number (the white corpuscles at the same time were 6,875 per cubic millimetre), was strong ground for confirming the diagnosis of congenital heart-disease.

V.—SPECIFIC GRAVITY OF THE BLOOD.

Lastly, *there are cases in which a determination of the specific gravity of the blood is of importance.*

The clinical symptoms of shock and of collapse are not easily distinguishable; but the pathology, and therefore the treatment, in these two conditions is very different. Expressed in as few words as possible, collapse results from excessive loss of fluid from the tissues, as, for example, after severe diarrhœa and vomiting in children; whereas shock is thought to depend upon paralysis of the vasomotor control, so that blood accumulates excessively in the splanchnic area. Collapse should be treated by replacing the fluids in the tissues by such methods as saline infusion; shock, on the other hand, by driving the excess of blood out from the splanchnic area, as by the "leg up and head down" position, and by bandaging the abdomen. There can be little doubt that many patients are infused when such procedure can do no good. An estimation of the specific gravity of the blood would indicate whether infusion should be resorted to or not. As is explained later, the determination is rapid and easy, and requires but a single drop of blood. If, as

in collapse, fluid has been drained from the system, the blood becomes more concentrated, and its specific gravity will be raised. If, as in shock, no fluid has been so drained away, but the blood has accumulated in the abdominal veins, the vessels nearer the surface will absorb water from the tissues, and the red corpuscles and albumen will be diluted, so that the specific gravity of the blood will be lowered. A raised specific gravity would be an indication for infusion, and for repeating the infusion many times, at intervals, as long as the specific gravity remained high.

VI.—METHODS OF EXAMINATION OF THE BLOOD.

And now to turn to the methods of a blood-examination.

First, as to the *method of making and staining blood-films*. It is most essential that the cover-glasses should be quite clean. If they are the least greasy, the film will not be a success. To clean the slips—and it is as easy to clean a thousand as a hundred—a simple and most efficient plan is to boil them in a solution of chromic acid. A small enamelled iron saucepan is suitable for the purpose; it should be half filled with water, and a tablespoonful of commercial chromic acid added. This dissolves readily; the water should be brought to the boiling-point, and then allowed to simmer gently. If vigorously boiled, the chromic acid splashes over. Chromic acid is better than nitric acid, because the latter gives off dense noxious fumes. Into the gently-simmering solution the cover-glasses are dropped *one by one*, to ensure the acid reaching them on both surfaces; and the whole is left simmering for half an hour. At the end of that time all grease will have been removed; the boiling is stopped; the chromic acid is washed away by a gentle stream of tap-water until no yellow colour is left between any two of the cover-slips; the tap-water is as far as possible poured off, and as much as possible of the remainder displaced by gently rinsing the whole mass of cover-slips with two or three changes of rectified spirit. Each cover-slip is then picked up individually with forceps, and transferred to a cylindrical glass jar filled with absolute alcohol. The absolute alcohol into which they are thus put will become contaminated with the residue of spirit on each cover-glass; so that, when the jar is full of cover-slips, the original absolute alcohol should

be poured off, and the vessel filled with fresh. It should then be stoppered, and the slips are ready for use. When one is needed, it is taken out with forceps ; the alcohol is burned off in the flame of a spirit-lamp, and the glass is left quite clean and dry. Should it splinter in the flame, it means that the alcohol is not quite "absolute," and the splintering ceases if that in the jar be poured off and fresh pure alcohol poured in.

The lobule of the ear is the best place from which to procure blood for an examination. The lobule is sometimes cleaned with ether first ; but warm water is as good, and then the ear should be carefully dried. Holding the lobule firmly at its base, between two fingers of the left hand, a quick deep stab is made with the needle, and the patient feels a very trifling pain ; this pain is made less obvious when the lobule is firmly held by the operator's left hand. The first drop of blood is wiped off, and the next may be used either for a film, or for counting corpuscles, or for hæmoglobin-estimation, or for determination of specific gravity ; and successive drops may be used for all four.

An excellent way of making a film is by means of a cigarette-paper. The latter is cut just narrower than the cover-slip ; a drop of blood is touched with one end, being lightly smeared along a thin line at the machine-cut edge of the paper ; the blood-smeared end is then laid, smeared surface downwards, upon one end of a cover-slip, and the cigarette-paper drawn quickly and steadily along the surface of the glass. A little practice will ensure the making of smooth, even films, and their thickness may be varied by varying the angle at which the cigarette-paper meets the cover-slip. The blood should dry upon the glass almost as quickly as the paper is drawn along ; otherwise the corpuscles run together in *rouleaux*.

The film having been made, the question arises as to how best to stain it. Many observers prefer Jenner's stain, because it both fixes and stains at the same time, and in a few minutes. Others prefer fixing the film by dry heat at 120° C. for two hours, and then staining with methylene blue and eosine. For differential counts some prefer Phear's stain, which is put upon the lobule of the ear ; the ear is then pricked through it, and a drop of mixed blood and stain examined

fresh, without making a film at all. But none of these methods are more certain in their results than the old-fashioned hæmatoxylin and eosine. The blood-film, as soon as it is dry, is dropped into a mixture of equal parts of absolute alcohol and ether. It is left for twenty-four hours, and next day is ready to stain. In another glass jar is kept some undiluted Delafield's hæmatoxylin, and in a third some watery eosine. The film, taken from the jar of alcohol and ether, is washed in water (preferably, but not necessarily, distilled); and then dropped into the jar of hæmatoxylin. In this way the stain is not wasted, but may be used again and again. The film is stained for five minutes, then washed in *tap*-water, which "blues" it; next dropped into the jar of eosine for thirty seconds; again washed in water, allowed to dry in the air after the excess of water has been removed with absorbent paper, and then mounted in Canada balsam. It is now ready for examination.

A rough observation may be made at once under the $\frac{1}{6}$-in. lens. If a differential count is needed, it is preferable to wait a little till the Canada balsam has somewhat set. For the differential leucocyte-count the $\frac{1}{12}$-in. lens. is very convenient, though the $\frac{1}{6}$-in. will do. The film should be moved continuously across the field from end to end by means of the mechanical stage; each leucocyte, as it is seen, should be noted down under its proper variety; and when the end of one row is reached, the film should be moved down exactly the width of one microscope-field. The next row is then counted right across the slide as before, and so on, until a sufficient number of white corpuscles have been counted. For a very rough enumeration, 100 may be enough; for accuracy, not less than 1,000; and even successive thousands, upon the same film, do not give identical proportions of the different leucocytes. It is a simple matter now to calculate, in percentages, the proportions of each. In health there are four easily distinguishable kinds of white corpuscles. The names given by different observers are very various, but the average proportions are:—

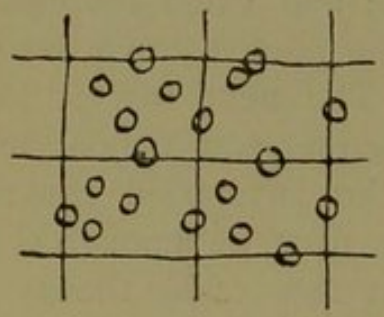
Small lymphocytes - -	about 25 per cent.
Large lymphocytes (or hyaline-leucocytes) - - -	„ 8 „

Polymorpho-nuclear cells (finely-granular eosinophile cells or neutrophile cells) - -	about 65 per cent.
Coarsely-granular eosinophile cells	„ 2 „

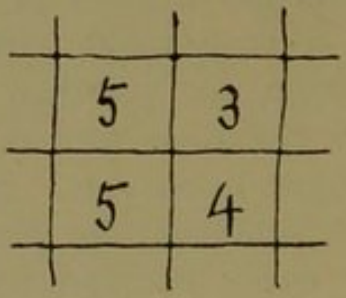
The chief difficulty, in counting a film, is in deciding whether certain of the cells should be classed as small or as large lymphocytes. Between the typical examples of these two varieties there are uncertain or intermediate forms. Such are usually counted as small lymphocytes.

As regards the enumeration of the red corpuscles little need be said. The Thoma-Zeiss apparatus is familiar, and is described in text-books. It is useful to know that, if blood be drawn into the pipette up to the mark 0·5, and Hayem's fluid[1] up to the mark 101, so that the dilution is 1 in 200, then the number of red corpuscles in every sixteen small squares is the *percentage* of red corpuscles; that is, with normal blood sixteen small squares should contain 100.

In order to ensure not counting the same corpuscle twice over, when it overlaps a cross line, it is well to regard any corpuscle as belonging to a square whenever it in any way touches the top or the right-hand line bounding that square; and as not belonging to the square when it touches the left-hand or bottom lines. For example, in the following diagram :—



the count is :—



If possible, sixteen sets of sixteen small squares should be counted, and the average taken of the whole number.

[1] Hayem's fluid is composed of :—Mercuric chloride, 0·25 grammes; sodium chloride, 0·5 grammes; sodium sulphate, 2·5 grammes; and distilled water, 100 cubic centimetres.

In counting the leucocytes it is better to use a dilution of 1 in 20, or of 1 in 10, and to count the leucocytes, not in the small squares, but in the large squares, each of which consists of sixteen small squares. They are so few in comparison with the red corpuscles, that a separate pipette and a smaller dilution are needed. In Thoma's fluid[1] the acetic acid lakes the red corpuscles, so that they disappear; and the leucocytes, stained by the methyl-violet, stand boldly out. At least sixteen of the big squares should be counted, and in doing this the mechanical stage is of great advantage.

The estimation of the hæmoglobin used to be a very unsatisfactory proceeding until very recently. One great difficulty was that the colour of the blood had to be compared with that of coloured glasses, or coloured solutions, such as carmine; the comparison varied in different lights, and the colour of the shed blood itself was rapidly altering during the time of comparison. Now, however, Haldane's hæmoglobinometer has obviated the difficulties of the older instruments, and reliable readings may be obtained with the greatest ease. The standard colour is a definite dilution of carboxy-hæmoglobin in a sealed tube, and the solution is quite permanent. By means of a small pipette a measured quantity of the patient's blood is obtained, and immediately mixed with distilled water in a graduated tube, so that the corpuscles are laked. The next proceeding is to convert the oxy-hæmoglobin into carboxy-hæmoglobin. This is readily done by means of coal-gas, introduced from an ordinary gas-burner through a brass U-tube provided for the purpose; and the carboxy-hæmoglobin derived from the patient's blood is diluted until the tint is exactly that of the standard carboxy-hæmoglobin in the sealed tube. The process occupies but a minute or two, and the comparison of one carboxy-hæmoglobin solution with another can be made, in any light, with accuracy. The percentage of hæmoglobin is then read off on the scale upon the mixing-tube. An additional advantage in this instrument is that there is a definite blood-standard; "normal" blood being such as yields 18 c.c. of oxygen from every 100 c.c. The apparatus is fully described in the *Journal of Physiology*, Vol. XXVI., 1901, p. 497.

[1] Thoma's fluid consists of a solution containing 0·3 per cent. of acetic acid in water, coloured with a few drops of methyl-violet.

Lastly, the specific gravity of the blood is easy to determine. Chloroform and benzene mix readily together; the specific gravity of the former is high, that of the latter is low. By mixing the two in different proportions a fluid of any intermediate specific gravity may be prepared. The best plan is to begin with a mixture of which the specific gravity is about 1,056, the average for normal blood. This is poured into an ordinary urine specimen-glass, or other suitable and sufficiently deep glass vessel. A drop of blood is drawn up into a fine glass pipette, such as that in the hæmoglobinometer; and the drop expelled into the mixture of chloroform and benzene. If it float, the specific gravity of the fluid is too high; more benzene must be added. If it sink, more chloroform. It is useful to have two specimen-glasses, so that the fluid, with the drop of blood, can be poured from one to the other, and complete mixture ensured. By adding a little more chloroform or a little more benzene according as the bead of blood sinks or floats, a point will rapidly be reached at which the drop neither sinks nor floats. Lest the droplet should have altered in specific gravity since it was placed in the mixture, a fresh drop may now be put in. If this neither sink nor float, the specific gravity of the mixture of chloroform and benzene, as read off upon the urinometer floated in it, will be the specific gravity of the blood.

A complete blood-examination may be carried out in the following order:—The ear is pricked. (1.) Blood-films are made and dropped into absolute alcohol and ether. (2.) Blood is drawn up into the red-corpuscle pipette to the mark 0·5, diluted to the mark 101 with Hayem's fluid, quickly mixed, and put aside to count presently. (3.) The same is done with blood in the white-corpuscle pipette. (4.) Blood is drawn up to the mark on the hæmoglobinometer pipette, expelled into a little distilled water in the graduated tube, and thus laked. (5.) Then another drop is taken in the same pipette, and put into the mixture of benzene and chloroform. The pipette should then be cleaned. This is quickly done by drawing distilled water up into it two or three times, blowing it out again each time; then in the same way drawing up absolute alcohol twice successively, to dehydrate the tube; and finally ether in the same way, to remove the alcohol, and to leave

the tube dry and clean when the ether evaporates, as it does in a few minutes. The determination of the specific gravity may then be completed; and the chloroform and benzene returned to a stoppered bottle for future use; the blood-drops are readily filtered out by pouring it through muslin. The hæmoglobin-estimation may then be continued and completed. Finally, after further complete mixing in the pipettes before samples are taken, the red and the white corpuscles are successively enumerated under the microscope. The films wait till next day, and then require but ten minutes for their staining and mounting.

The complete blood-examination, without a differential leucocyte-count, need not occupy more than three-quarters of an hour; a differential count will make the time required a little longer. In many cases a partial examination is all that is required. In one, for example, only the specific gravity; in another, only the total number of leucocytes; in another, a differential count of the leucocytes. Such a partial examination requires comparatively little time; how extremely valuable it may be in certain instances, and under widely differing circumstances, has been emphasised by the cases already quoted.